Microbes and Alzheimer's Disease: New Findings Call for a Paradigm Change

Microbes and Alzheimer's Disease: New Findings Call for a Paradigm Change

Jürgen G. Haas[1,*] and Richard Lathe[1,*]

[1]Division of Infection and Pathway Medicine, University of Edinburgh, Little France Crescent, Edinburgh, UK

*Correspondence: juergen.haas@ed.ac.uk (J. Haas); richardlathe@ed.ac.uk (R. Lathe).

Keywords: neurodegeneration; antimicrobial protein; herpesvirus; HSV-1; HHV-6; HHV-7.

References: 10

Text wordcount: 1820

Abstract: 68

Items: 1 Figure

Abstract

Two papers in *Neuron* provide compelling new indications of a link between herpesviruses and Alzheimer's disease (AD). Readhead *et al.* report an increased abundance of human herpesvirus 6A and 7 (HHV-6A/7) in AD brain, whereas Eimar *et al.* show that binding of the AD signature protein, Aβ, to herpes simplex virus type 1 (HSV-1) and HHV-6 surface glycoproteins causes fibrillar Aβ agglutination that can protect against viral challenge.

Main text

Two new papers in *Neuron*, by Readhead *et al.* [1] and Eimer *et al.* [2], provide fresh impetus to the link between infection and Alzheimer disease (AD). A causal association has long been suspected [3], underlined by escalating data indicating that a key signature of AD – amyloid-ß (Aß) – can act as an antimicrobial defense peptide [4].

Because the majority of the adult population already harbors subclinical infections with several herpesviruses, including HSV-1, HHV-6/7 and Epstein–Barr virus (EBV), it seems plausible that a declining immune system with age might permit reactivation of erstwhile silent viruses, and thereby exacerbate pathology stemming from other causes [4]. An alternative hypothesis, however – which recent studies seem to reinforce – places viral infection as a more central player, arguing that viruses such as those mentioned above are essential triggers of the pathophysiology.

Herpesviruses: Evidence for Causation

To place these recent developments in context, it should be mentioned that evidence has recently emerged supporting a causal link between AD development and infection with HSV-1, and possibly HSV-2. Briefly, based on retrospective analyses, population data from Taiwan argue that 10 year AD development in patients with severe herpesvirus infection could be prevented, in ~90% of treated patients, by aggressive antiviral medication at time of the infection [5,6]. It should be underscored that the treated population in this study was limited to subjects severely affected by

HSV. Nevertheless, this study firmly demonstrates, we would argue, that herpesviruses can indeed cause AD, in line with earlier pioneering work by Ruth Itzhaki and others ([3] and references therein). Two key questions arise, however: (i) which of the different herpesvirus species are relevant for the emergence of AD? – and (ii) in the broader picture of AD across the population, are the herpesvirus-linked cases exceptional ones, or is the microbial connection a more general feature of the disease? The paper by Readhead *et al.* [1] tackles both questions head-on.

Over-Representation of Human Herpesviruses in AD

To date, most studies on herpesviruses in AD have focused on HSV-1. HSV-1 DNA is widely found both in AD brain and in control tissue. In AD brain, an association was demonstrated between HSV1 and Aß plaques. By contrast, herpesviruses such as HHV-6 have been so far predominantly implicated in multiple sclerosis, but (to our knowledge) not in AD. The striking new results from Readhead *et al.* [1] cut across this compartmentalization.

The team led by Joel Dudley, based at Mount Sinai, New York, started out by looking for possible commonalities that might underpin the spectrum of gene regulation changes seen in AD brain. Unexpectedly, this analysis highlighted transcription factors such as C2H2-TF, and also G-quadruplex (G4) motifs, that are known to be involved in regulating viral transcription. The researchers then inspected AD brain samples for 515 known human viruses. Strikingly, they discovered that transcripts of specific herpesviruses are increased in AD brain – predominantly HHV-6A and HHV-7, although there was also evidence of over-representation of HSV-1 and HSV-2

transcripts. These patterns held up when three different banks of AD samples were inspected (Figure 1). Notably, the overabundance was not restricted to a few exceptional cases, but from the data at hand appears to be a more general feature of AD. Other potential AD-linked pathogens including spirochetes (as proposed by Miklossy and others) were not surveyed; further work in this direction will be essential.

The researchers also found increased abundance of HHV-6A (and HSV-2) DNA, in line with earlier work [7], pointing to active viral replication in AD brain. Moreover, the presence of HHV-6A and HHV-7 was significantly associated with severity of dementia and brain pathology. In further support of a link, genetic variants linked to infection with these viruses overlapped with genes known to be involved in host–virus interactions and panels of 'AD risk' genes. Of note, similarly to HSV-1/2, HHV-6 and -7 are well-known causes of viral encephalitis, in particular in immunocompromised individuals, and have also been shown to be associated with demyelinating brain diseases.

The AD Signature Protein Aß Targets Herpesviruses

In the same issue of *Neuron*, Rob Moir, Rudy Tanzi, and colleagues at Harvard [2], extending their earlier seminal findings that the AD signature protein Aβ is an antimicrobial peptide [4] (independently validated by researchers at Sherbrooke and elsewhere), now confirm that Aβ binds to HSV-1 and HHV-6 surface glycoproteins, and causes fibrillar agglutination and protection against virus challenge, further reinforcing the link between herpesviruses, Aß, and AD.

Drivers or Passengers? Differential Tropism Could Point the Way Forward

Readhead *et al.* highlight the challenge ahead: "Distinguishing the earliest drivers of disease from the 'opportunistic passengers' of a multi-decade neurodegenerative process is especially formidable..." [1]. In other words, herpesvirus infection of key degenerating brain tissues in clinical AD might either be a causal component/cofactor of the disease or, alternatively, represent opportunistic invasion of brain tissues that are damaged early in AD progression. An important consideration is the frequently observed invasion of inflammatory immune cells into brain areas most prominently affected by AD. As discussed next, the differential tropism of the viruses might offer an insight.

HSV-1 and HSV-2 are considered to be 'neurotropic', in that they have a predilection to infect and replicate in neurons. By contrast, the HHVs as a group have generally been dubbed 'lymphotropic', in that they principally target immune cells, including T cells and macrophages. HHVs have also been reported to infect glial cells (e.g., oligodendrocytes). Viral tissue tropism can be ascribed mainly – but not exclusively – to the expression of their cellular receptors. Work has been done on identifying receptors for HSV-1 and mapping them in the human brain (e.g., [8]), but much less is known about receptors and coreceptors for HHVs. Overall, based on the existing data, it seems that the dichotomy of neuro- versus lymphotropic viruses is in fact inaccurate, although it serves well to illustrate potential reciprocal interactions between HHV-6A/B, HHV-7, and HSV-1/2 *in vivo*, by highlighting their action via different pathways, and potentially differential contributions of different herpesvirus species.

AD is accompanied by a major CNS influx of proinflammatory immune cells, including macrophages as well as T and B cells. Accordingly, it is conceivable that invading cells harboring episomal or integrated HHV genomes might have skewed the AD versus non-AD ratio of viral transcripts detected by Readhead *et al.* [1]. With that in mind, it would be very interesting to study the distribution of HHV genomes in neuronal versus non-neuronal cells.

In addition, active herpesvirus infections can foster reactivation of other latent herpesviruses: for example, human cytomegalovirus infection can be accompanied by reactivation of latent HSV-1. In this regard, a 2016 paper by Chapenko *et al.* [9] is notable. Briefly, in this study on unspecified encephalopathy (UCP), some 30–80% of all human brain samples (controls and UCP patients) were positive for both HHV-6 and HHV-7, and there was no significant difference in positivity between controls and UCP patients. However, there was a highly significant (roughly 100-fold) increase in HHV-6 genome content in the frontal and temporal lobes of UCP patients [9]. Despite diagnostic caveats, it seems clear that some event (or events) in these individuals switched HHV replication on – perhaps by genome reactivation induced by another triggering factor/agent, or by an influx of susceptible cells, or possibly both.

Conclusions: Rethinking AD from Scratch

Immense effort has been expended on targeting Aß, based on the overall premise of Aß being a key driver of the pathophysiology of the disease. Despite significant resource investment, and clinical testing of a large number of compounds, this

approach has so far been unsuccessful. The realization that Aß can act as a crucial defense molecule calls for a reformation of its role in the disease, and – we think – reconsideration of the priorities in exploring possible treatments strategies (or preventive measures). The papers by Readhead *et al.* [1] and Eimer *et al.* [2], as well as Tzeng *et al.* [5] and others, have brought herpesviruses (and other pathogens) to the fore as vital contributors to AD development. But obviously, questions remain. For instance, how broad is the set of pathogens that can be linked to AD? And are they merely opportunist infections of a degenerating brain?

Retrospective epidemiological studies linking HSV-1 and AD [5,6], as discussed earlier, coupled with a recent study by Rathore *et al.* at Genentech reporting that genetic variants in PILRA, a receptor for HSV-1 glycoprotein B (and that affect HSV-1 viral entry into cells), are associated with AD [10], do indicate that HSV-1 may play a direct role in disease development. Interestingly, other potential HSV receptor genes are located within the *APOE* locus, an observation which could offer a new perspective on some of the linkages between *APOE* variants and AD. Regardless, however, it could well be that the role of HSV-1 in AD pathology is more complex than it seems. It is possible, for instance, that HSV-1 inevitably brings in HHVs, by immune cell recruitment and/or reactivation, leading to 'double pathology'. The Readhead *et al.* paper [1] is a convincing demonstration that HHVs are also likely to play a role.

But what is it that first triggers AD? It does not seem to be infection *per se*, because many of the viruses discussed above are fairly prevalent, and are found in many subjects who do not eventually develop AD. Key 'AD genes' encoding immune system modulators such as APOE are clearly important (and *APOE* alleles modulate

susceptibility to diverse pathogens including herpesviruses, HIV, *Chlamydia*, and malaria); lifestyle factors such as stress may play a role as well. Squinting ahead, it could be that a combination of infection, genes, age, and environment might explain a majority of AD cases.

However, lest we spend too much time peering into the mist, priorities in searching for cures should be focused – we think – on what we do know at this point. As it stands, the field has established that viruses are somewhere central in the causal chain, at least in a subset of AD patients. We believe that the increasing evidence over the past few years – including the papers by Readhead *et al.* and Eimer *et al.* – that chronic infections and defense mechanisms including inflammatory processes are central to AD, warrants revisiting antiviral drugs such as aciclovir (and possibly also vaccination) as potential routes to combating AD.

As a final note, whereas herpesviruses have emerged as a recurring theme in several recent studies on AD, other pathogenic candidates have been identified as well, and future work should not be solely limited to herpesviruses. Both spirochetes and fungi, for instance, have been previously associated with AD. And in their study, Readhead *et al.* detected traces of diverse pathogens such as human adenovirus, Ippy arenavirus, Torque teno virus, and Kaposi sarcoma-associated herpesvirus in AD brain. More broadly, it would be important, we think, to examine the possible role of pathogens in the etiology of other neurodegenerative diseases, including Parkinson's disease, where an infectious component has long been speculated.

Conflict of Interest Statement

R.L. has previously acted as a consultant to industry in the field of AD.

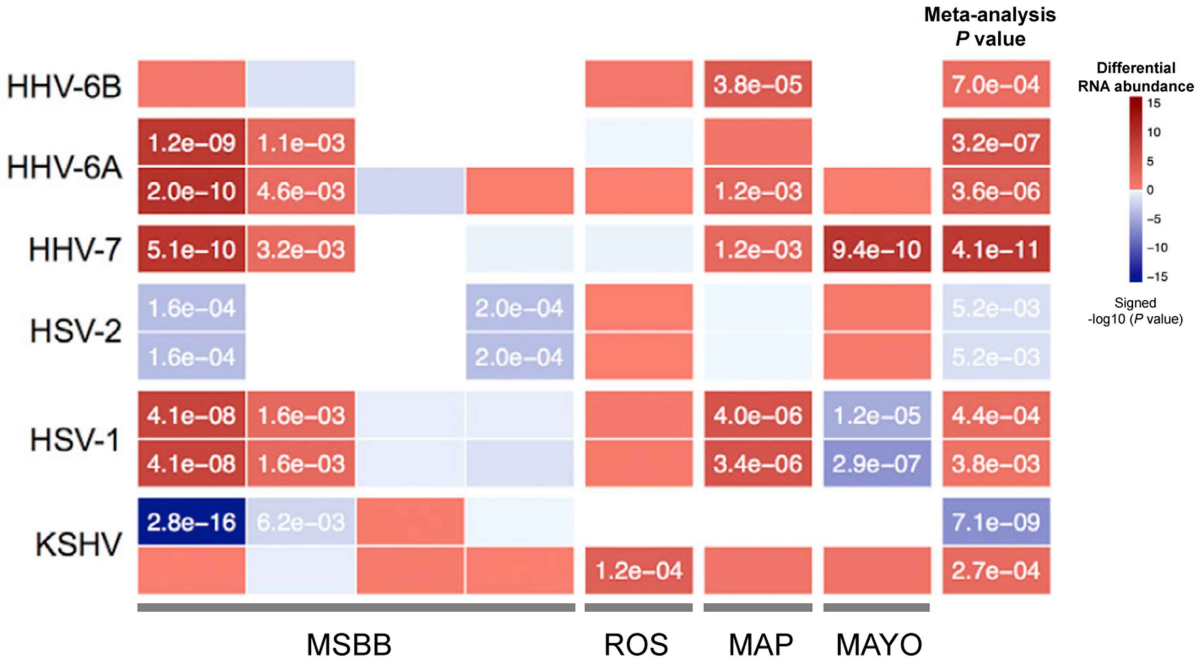

Figure 1. Differential Abundance of Viral Transcripts in Alzheimer's Disease (AD) Versus Control Brain Samples. Figure adapted, with permission, from Figure 2 of Readhead *et al.* [1]. Abbreviations: KSHV, Kaposi sarcoma associated herpesvirus; MAP, Memory and Aging Project (prefrontal cortex); MAYO, Mayo Clinic Brain Bank (temporal cortex); MSBB, Mount Sinai Brain Bank (cortex); ROS, Religious Orders Study (prefrontal cortex).

References

1 Readhead, B. *et al.* (2018) Multiscale analysis of independent Alzheimer's cohorts finds disruption of molecular, genetic, and clinical networks by human herpesvirus. *Neuron.* Published online June 21, 2018. http://dx.doi.org/10.1016/j.neuron.2018.05.023.

2 Eimer, W.A. *et al.* (2018) Alzheimer's disease-associated beta-amyloid is rapidly seeded by herpesviridae to protect against brain infection. *Neuron* (in press)

3 Itzhaki, R.F. *et al.* (2016) Microbes and Alzheimer's disease. *J. Alzheimers. Dis.* 51, 979–984

4 Kumar, D.K. *et al.* (2016) Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci. Transl. Med.* 8, 340ra72

5 Tzeng, N-S. *et al.* (2018) Antiherpetic medications and reduced risk of dementia in patients with herpes simplex virus infections – a nationwide population-based cohort study in Taiwan. *Neurotherapeutics* 15, 417–429

6 Itzhaki, R.F. and Lathe, R. (2018) Herpes viruses and senile dementia: first population evidence for a causal link. *J. Alzheimers Dis.* 64, 363–366

7 Lin, W.R. *et al.* (2002) Herpesviruses in brain and Alzheimer's disease. *J. Pathol.* 197, 395–402

8 Lathe, R. and Haas, J.G. (2017) Distribution of cellular HSV-1 receptor expression in human brain. *J. Neurovirol.* 23, 376–384

9 Chapenko, S. *et al.* (2016) Detection frequency of human herpesviruses-6A, -6B, and -7 genomic sequences in central nervous system DNA samples from

post-mortem individuals with unspecified encephalopathy. *J. Neurovirol.* 22, 488–497

10 Rathore, N. *et al.* (2018) Paired immunoglobulin-like type 2 receptor alpha G78R variant alters ligand binding and confers protection to Alzheimer's disease. *BioRxiv.* Published online May 18, 2018. http://dx.doi.org/10.1101/325936